Evidence for the unique function of DHA during the evolution of the modern hominid brain

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Summary : The African savanna ecosystem of the large mammals and primates was associated with a dramatic decline in relative brain capacity. This reduction happened to be associated with a decline in docosahexaenoic acid (DHA) from the food chain. DHA is required for brain structures and growth. The biochemistry implies that the expansion of the human brain required a plentiful source of preformed DHA. The richest source of DHA is the marine food chain while the savannah environment offers very little of it. Consequently *H. sapiens* could not have evolved on the savannahs. Recent fossil evidence indicates that the lacustrine and marine food chain was being extensively exploited at the time cerebral expansion took place and suggests the alternative that the transition from the archaic to modern humans took place at the land\\water interface. Contemporary data on tropical lake shore dwellers reaffirms the above view. Lacustrine habitats provide nutritional support for the vascular system, the development of which would have been a prerequisite for cerebral expansion. Both arachidonic acid (AA) and DHA would have been freely available from such habitats providing the double stimulus of preformed acyl components for the developing blood vessels and brain. The w3 docosapentaenoic acid precursor (w3DPA) was the major w3 metabolite in the savanna mammals. Despite this abundance, neither it or the corresponding w6DPA were used for the photoreceptor nor the synapse. A substantial difference between DHA and other fatty acids is required to explain this high specificity. Studies on fluidity and other mechanical features of cell membranes have not revealed a difference of such magnitude between even a-linolenic acid (LNA) and DHA sufficient to explain the exclusive use of DHA. We suggest that the evolution of the large human brain depended on a rich source of DHA from the land\\water interface. We review a number of proposals for the possible influence of DHA on physical properties of the brain that are essential for its function.

Keywords : arachidonic, brain, blood vessels, docosahexaenoic, evolution, lacustrine, marine foods, membranes, nutrition, Nyasa, Turkana, Australopithecus, H. erectus, H. sapiens

ARTICLE

The invertbrates, reptiles and mammals

The origin of air breathing animals

For the first 2.5 billion years of life on the planet, the blue-green algae dominated the proto oceans. The photosynthesis of the algae produced complex molecules including proteins, carbohydrates and lipids which were rich in w3 fatty acids. Based on the explosion of the phyla in the fossil record, oxidative metabolism became predominant about 600 million years ago. Thus animals, visual, nervous systems and brains evolved in a DHA rich environment [1]. DHA is the most prominent essential fatty acid used for the structures and functions of the photoreceptor and synaptic junction. So the question addressed in this paper is why, and what are the evolutionary implications of the abundance of DHA in the marine food chain compared the relatively paucity in land ecosystems.

Mammals

The dominance of w3 fatty acids in the early oceans was associated with fish and reptiles requiring w3 fatty acids for their reproduction. This dominance persisted until the end of the Cretaceous period, 70 million years ago. In the wake of the extinction of the giant reptiles, cycads, ferns and their allies, the flowering plants appear in the fossil record.

They stored lipids, for energy during germination, containing seed oils rich in w6 fatty acids. Then, a new set of species, the mammals, evolved : it may not be a coincidence that they required w6 fatty acids for their reproduction.

Mammalian brain size is larger in relation to body size compared to the previous egg laying amphibians, reptiles and fish. The difference could be explained by the evolution of the placenta. The placenta enables nutrients and energy to be focused continuously on the development of one or a small number of progeny throughout the critical time of brain development. In the human, 70 % of the calories transferred by the placenta to the fetus is devoted to brain growth. The placenta is a rapidly growing vascular system with a high requirement for w6 fatty acids especially AA. In 42 species so far studied, AA and DHA are major acyl constituents with the precursors being virtually absent. So the emergence of the w6 fatty acids may have added the missing biochemical link, liberating genetic potentials for vascular development and hence the evolution of the placenta, mammary gland and the larger brains of the mammals.

The difference between species is not the chemistry but the extent or size to which the brain is developed [1]. The manner of these differences is so large as to imply the availability of AA and DHA were limiting factors in the evolution of the brain [1, 2]. Indeed, the need for both w6 and w3 fatty acids for development and health of the vascular system and brain has long been recognised [3].

Evidence from paleontology

The evolution of the modern human brain

The accepted dogma regarding the evolution of *Homo sapiens* is that he was originally a hunter and gatherer on the African savanna. A study of savanna and other African species show that as they evolved larger and larger bodies, the relative size of the brain diminished logarithmically with

increase in body weight [1, 4]. A cebus monkey of 0.9 kg body weight has 2.3 % of its body weight as brain, a 60 kg chimpanzee 0.5 %. The larger gorilla at 110 kg has only 0.25 % brain which is physically smaller than the chimpanzee's brain. At the extreme, the one ton rhinoceros has < 0.1 % with its brain weighing only 350 g. It reaches that massive one ton body weight at four years of age. Why does size and velocity of growth matter ? The reason is that the biosynthesis of AA and DHA is relatively slow, and may not be able to keep pace with body growth in fastgrowing animals. Rats and mice desaturate and chain elongate the parent essential fatty acids to produce larger amounts of AA and DHA than their precursors. Stepping up in size from the guinea pig to the wild pig the impact of velocity of growth results in a progressive decline in AA and DHA whilst the precursors linoleic and a-linolenic acids become more dominant in liver lipids [1]. Instead of DHA, the w3DPA is now the major metabolite of a-linolenic acid [5].

So the faster an animal grows, the larger it becomes and the greater is the constraint of the biosynthesis of AA and DHA. The large savanna, mammals of Africa all shared the same fate namely, DHA and brain capacity declined as body size accelerated. The important issue is that desaturation and elongation in these large mammals peters out at the w3DPA with relatively little DHA being synthesized. What little DHA is synthesized is used in the brain and photoreceptor. The abundant w3DPA is not found here. Brain size was sacrificed not brain DHA [1, 6]. This fact raises two issues :

– The savanna food chain on which *Homo sapiens* is supposed to have evolved is fairlydevoid of DHA so how did Homo evolve a large brain ?
– Why was the more readily available w3DPA not used for the brain instead of DHA ?

Modern human intellect and brain-specific nutrition

Australopithecus spp. are unremarkable in their apparent encephalization throughout their evolutionary history as far as can be deduced from the fossil record. No australopithecine has a cranial capacity much over 500 cm3 [7], despite the existence of the genus for over 3 Myr. Contrast this to genus Homo, whose cranial capacity doubled from *H. erectus* to *H. sapiens* in a span of at most 1 Myr *(table 1)*. The *Homo spp.* fossil evidence and encephalization quotient (EQ) values do not support a slow, linear Darwinian progession towards modern intelligence, but rather a sudden, exponential growth of relative brain size in the last 200,000 years or so.

Table 1. *Mean brain volumes and encephalization quotients (EQ) for selected hominoid species. EQ1 from the calculations of Martin [4] ; EQ2 from the calculations of Harvey and Clutton-Brock [47].*

Species	**Brainvolume (cm3)**	**EQ1**	**EQ2**
A. afarensis	384	1.23	1.45
A. africanus	420	1.31	1.62
A. boisei	488	1.37	1.72
A. robustus	502	1.49	1.92

H. habilis	579-597	1.74-1.79	2.10-2.29
H. rudolfensis	709	1.41	2.11
H. erectus	820-844	1.59-1.63	2.38-2.44
H. sapiens	1250	3.05	4.26
P. troglodytes	410	1.25	1.57

The earliest evidence for modern *H. sapiens* is found in Africa. *Homo spp.* in general are associated with lake shore (lacustrine) environments in the East African Rift Valley, while Australopithecines are associated more with forested areas [8, 9]. Thus far, evidence for precocious cultural development of *Homo sapiens* is exclusively confined to lacustrine and coastal marine environments. Lakeshore sites in the Rift Valley have yielded fairly sophisticated stone tools as old as 260 kyr associated with *H. sapiens* remains. The implications of this land/water habitat providing brain specific nutrients has largely been overlooked. Fossils from coastal sites on the southern Cape of South Africa are widely regarded as the earliest modern human fossils [10, 11, 12]. At numerous sites along the Cape, hominid occupation is evidenced dating from 120 to 60 kyrs before now. Modern human fossils dating to about 100 kyr have been recovered at Klasies River Mouth and Border Cave are found associated with incontrovertible evidence for the consumption of seafoods dating to the time of rapid cerebral expansion [10, 13, 14, 15]. Parkington [15] points out that in coastal hunter-gatherer cultures, women are responsible for collecting shellfish. So stone age women could have easily provided themselves with a plentiful source of brain-specific nutrition, even when strength/mobility are compromised during pregnancy and lactation. Children would have naturally participated in exploitation of, at that time, this extremely rich resource. Early consumption of shellfish is also present in the archaeological record on the Mediterranean coast of Africa. It is likely to have occurred elsewhere as well, however most possible coastal sites which could be investigated have been obliterated by the higher sea levels of the current interglacial era.

Successful early Homo spp. were Tropical Coastal Migrants

Both *Homo erectus* and *H. sapiens* sucessfully colonized areas outside of Africa. There is all but unanimous agreement amongst paleoanthropologists that *H. sapiens* originated in Africa and then spread throughout the world [16,17, 18, 19]. Recently, stone tools of 0.8- 0.9 Myr have been found on the island of Flores, one of the Wallacean islands lying between Java and Timor in Indonesia [20]. The antiquity of the specimens suggests they were manufactured by *Homo erectus*, not *H. sapiens*. Although Java and Bali were periodically connected to the mainland during the Pleistocene glaciation, even at times of lowest sea level, reaching Flores would have required a sea crossing of at least 19 km. This implies that at least in Indonesia, *H. erectus* had already reached the cognitive capability to build and use watercraft repeatedly. Previous to Morwood *et al.* [20] recent discovery, the earliest evidence for the use of watercraft dates from about only 40 kyr or slightly earlier with the migration of *H. sapiens* from the Wallacean Islands to Australia. That initial colonization of Australia, Tasmania and New Guinea was accomplished by modern *H. sapiens*. Similar to the

movements of *H. erectus*, these early migrants are considered to have followed a tropical coastal route. Therefore, both the earliest occurrence of modern *H. sapiens*, the earliest use of watercraft and successful colonization of Southeast Asia were intimately associated coastal migrations and with the utilization of food resources from the marine food chain. We consider this association not accidental nor coincidental, but a reflection of the dramatic influence of brain specific nutrition on the evolutionary process. We do not accept the postulate that *H. sapiens a priori* evolved a large, complex brain, then began to hunt in order to maintain it – the brain must come first. Our thesis is that there must have been enough long chain polyunsaturated fatty acids (LCPUFA) available in the diet to :

1. Provide many generations of hominids with fuel for fetal/infant development as well as childhood and adult needs for the cardio-vascular system and the brain.

2. Allow for substantial amounts of 18 carbon polyunsaturated fatty acids (PUFA) which would have been oxidised for energy requirements [21, 22].

3. Explain and allow for our inefficient conversion of LA to AA and LNA to DHA (which is illustrated by preferential incorporation of DHA in the infant brain [23] and improved problem solving in infants fed DHA which persisted beyond the period of supplementation [24]).

The evidence on the extensive coastal and lacustrine exploitation implies LC-PUFA were consistently abundant in the food supply as we evolved. Homo did not however, go as far as the obligate carnivores in which the desaturation process is barely detectable [25]. If *H. sapiens* had developed his intellect by evolving into a primate which can make heroically efficient use of 16 and 18 carbon PUFA from vegetarian sources, we would see an obvious signature in our current PUFA metabolism, since we are only a 300-100 kyr old species. Instead we see the opposite. We might hypothesize that *Australopithecus spp.* could not mount this heroic metabolic effort either which explains why their brain capacity was constrained by their land based diet at 400-500 cc for 3 Myr and explains why exploitation of coastal foods fits with the rapid and recent cerebral expansion to 1.3 kg after 3 Myr of a static brain size [9, 15].

Contemporary evidence

The human brain requires a rapidly developing heart and vascular system to meet the prodigious energy and nutrient demand during its development. The vascular system itself has a high requirement for AA [26]. Hence the principles of vascular development were *sine qua non* vital for the final evolution of the large human brain. If we now examine the contemporary evidence on cardiovascular disease we find that land based animal fats have been causaly linked to heart disease as revealed by the Seven Countries study of the 1950s and even earlier [27]. Saturated fats and vascular degeneration would be incompatible with CNS expansion. Also, there is increasing evidence that cardio-vascular disease has its origin in poor fetal nutrition [28] consistent with our hypothesis of long term, multi-generation effects operating on vascular and CNS evolution. Those forces can act for expansion or degeneration. Worldwide diets and cardiovascular risk factors show that marine fats, especially DHA, are cardioprotective [29, 30, 31]. It is well known that people living on sea foods have low cardiovascular risk factors. The diet of contemporary populations beside East African lakes (Lake Nyasa and Lake Turkana) is still largely based on fish rich in w3 and w6 LCPUFA. From *table 2*, calculated intakes of w3 LC-PUFA are 1-4 g/d and AA 0.5-1.0 g/d, compared with w3 LC-PUFA 0.2-

0.3 g/d and AA 0.1-0.2 g/d for populations consuming Western diets. Total dietary fat in the African populations is similar at 10-15 % of the dietary energy [32].

Table 2. *The FA composition data of the fish from Lake Nyasa and Turkana (wt % of fatty acids)*

	Turkana Tilapia	**Turkana Perch**	**Nyasa Mfui**	**Nyasa Kambale**	**Nyasa Carp**	**Nyasa Mbelele cat fish**
Fat g % wet weight	2.3	2.6	1.1	1.8	4.9	10.3
20 : 4w6	8.4	7.7	8.0	5.8	5.8	4.3
20 :5w3	2.8	1.8	3.1	2.2	1.8	4.2
22 :5w3	3.2	3.8	3.7	5.2	5.0	1.8
22 :6w3	15.7	18.1	19.1	13.3	7.8	8.6

We have compared East African lake shore, mainly vegetarian and Europeans cardiovascular risk factors *(table 3)*. Blood cholesterol, blood pressure, lipoproteins (Lp(a)) are lower in the contemporary Africans living on lake shores of Turkana and Nyasa compared to their vegetarian cousins and Europeans. Plasma AA, eicosapentaenoic acid and DHA are highest in the fish eating, lake shore people and least in the vegetarian or omnivorous inland cousins. Furthermore, comparison of children from the lake shore versus European children living in East Africa showed that the two populations can be separated on the basis of blood cholesterol at the age of 6 years ! Whilst the European children's blood pressure and cholesterol continues to rise the Africans remain stable illustrating the compatibility of the lacustrine diet with good cardiovascular performance and the needs of fetal brain expansion. It is of interest that the Turkana have the highest mitochondrial DNA diversity of any ethnic group. In fact 36 Turkana people have a higher diversity than the world-wide population database. The simplest interpretation is that humans date back to the East African Rift Valley [33].

Table 3. *Comparison of cardiovascular risk fatty factors, plasma and fish fatty acid composition of lake shore, fish eating vegetarian and European, communities in the Rift Valley and East Africa.*

Populations	**Largely vegetarian**	**Lake shore**	**Significance of differences**
Plasma lipids (mg/dL)			P
Plasma	TC 136. ± 39.8 n = 686	122 ± 30.9 n = 622	< 0.05

Plasma TG	105 ± 53.1	80.6 ± 40.7	< 0.001
Lp(a)	32.3 ± 22.4	19.9 ± 17.7	< 0.001
Blood pressure (mmHg)			
Nyasa			
Systolic	135 ± 20.4	120 ± 15.1	< 0.001
Diastolic	77.6 ± 10.6	70.5 ± 8.9	< 0.001
Turkana			
Systolic	European	El Molo	
Age yrs			
0-3	82 ± 14 n = 15	85 ± 9.7 n = 6	ns
6-10	98 ± 22 n = 16	87 ± 17 n = 16	ns
16-20	90 ± 28 n = 24	119 ± 28 n = 14	< 0.001
25-45	31 ± 34 n = 265	105 ± 30 n = 24	< 0.001
Cholesterol mg/dl			
0-3	102 ± 24 n = 15	97 ± 18 n = 12	ns
6-8	167 ± 35 n = 18	112 ± 32 n = 16	< 0.01
25 +	228 ± 44 n = 145	147 ± 49 n = 24	< 0.001
Plasma FA (wt %)			
Lake Nyasa			
LA	14.8 ± 4.30 n = 53	23.9 ± 4.37 n = 53	< 0.002
LNA	0.60 ± 0.20	0.31 ± 0.14	< 0.001
AA	8.26 ± 1.94	9.85 ± 2.68	< 0.005
EPA	0.72 ± 0.22	2.48 ± 1.35	< 0.001

DHA	1.48 ± 1.04	5.93 ± 1.77	< 0.001
Lake Turkana	El Molo* n = 32	Bantu n = 98	European n = 124
LA	9.3 ± 3.0	22.8 ± 4.8	19 ± 4.9
DHLA	1.9 ± 0.7	3.5 ± 1.3	2.4 ± 1.1
AA	12.2 ± 3.8	5.1 ± 2.7	7.0 ± 2.4
EPA	4.7 ± 1.3	1.6 ± 0.8	0.5 ± 0.2
DPA	2.6 ± 0.9	3.2 ± 1.2	2.1 ± 0.9
DHA	9.3 ± 3.3	3.5 ± 1.3	5.6 ± 2.2

* $p < 0.001$ for LA, AA & DHA in El Molo cfd all others. N, number of subjects ; TC = Total Cholesterol ; TG, Triglycerides. LA, Linoleic Acid ; LNA, alpha Linolenic Acid ; AA, Arachidonic Acid ; EPA, Eicosapentaenoic acid ; DHA, docosahexaenoic acid. Values are the average ± SD. Adapted from ref. [32] : El Molo live on a lava desert which runs down to the eastern shore of Lake Turkana, N. Kenya [48], The Bantu were Baganda and Bunyoro people of central Uganda, The Europeans were living in East Africa mainly, Kampala Uganda (data from 49). The slow conversion of linoleic to AA and a-linolenic to DHA is illustrated in the equilibrium of the higher linoleic acid content of the plasma lipids and the lower AA and DHA in the vegetarian and European plasmas compared to the fish eating people where preformed AA and DHA is consumed in the duiet and appears as higher levels in the plasma. Such data reflects the rate limitations of the conversion process especially D-6 desaturase which is involved twice in the synthesis of DHA [50]. The higher circulating levels of AA and DHA would favour their incorporation into the developing brain where their incorporation is an order of magnitude greater than their synthesis from precursors [51].

These unique conditions of the Rift Valley lake shores replicate the contrast in the high mortality from vascular disease and high prevalence of mental disease amongst US and Europeans versus the low risk of Japanese, Greenland and Inuit Indians living on a rich fish and sea food diet [34]. Similarly, comparison of fish eating populations in the Faroes compared to their genetically similar mainland Denmark contemporaries, shows that the high intake of fish and seafoods results in higher birthweights and a lower proportion of preterm deliveries [35]. The advantage of longer gestations is the greater exposure of the fetus and its developing vascular system and brain, to the placental biomagnification of AA and DHA [36]. The conclusion is that land based diets led increasingly in this century to cardiovascular disease being no. 1 killer in Western consumers which would have made cerebrovascular expansion in utero difficult, and been incompatible with expansion of the hominid brain.

Experimental support for the above case came in an unexpected result from studies on diabetes by our colleagues, Professor Lucilla Poston and others at St Thomas's Hospital Medical School. Pregnant rats were subjected to high saturated fat diets similar to those consumed in Western countries and blood vessel function tested in mothers and newborn offspring. The results from small vessel myography described arterial dysfunction specifically associated with the high, fat Western type diet. Vascular function tests on the 15 day old pups from mothers on the high (30 %) fat diet exhibited reduced vascular endothelium dependent relaxation to acetylcholine (ACh) with evidence of constrictor responses to noradrenaline and the thromboxane mimetic U46619. The vascular dysfunction was still observable at 120 days of age despite rearing on a normal diet. Thus the high fat diet fed to the mother changed the intrauterine milieu which caused persistent vascular dysfunction in the newborn animals without any genetic predisposition. Diabetes imposed on the high fat diet made vascular function worse. Biochemical analysis of the tissues from the control low fat and high saturated fat animals revealed the high fat diet selectively depressed liver DHA of the pups. Here is experimental evidence of the negative influence of land based animal fats fed to the mother on the next generation, emphasising the importance of long term nutritional forces [37].

The specificity of DHA

The question which arises from this discussion is what is so special about DHA ? Why has DHA been chosen so overwhelmingly for photoreceptor and synaptic membranes, despite the availability of similar molecules which would be less difficult to obtain, and are less vulnerable to oxidative damage [38, 39] ? In particular, what advantage does it convey relative to the very closely related w3 and w6 DPAs, each of which differs from DHA only in the absence of one double bond (between carbons 4 -5, and 19-20, respectively) ? As described above, Nature's preference for DHA in the brain is strikingly demonstrated in large land mammals, in which DPA is the dominant w3 metabolite yet neural membranes still retain the DHA-rich composition observed in other species (possibly at the expense of gross brain size, since DHA is in such limited supply). Significant quantities of the w6 form of DPA are observed only in situations of artificial w3 deficiency, yet even here brain membranes are resistant to decreases in their DHA levels. Nature is thus highly sensitive to the slight difference between DHA and DPA molecules ; the presence of DHA's full complement of six double bonds is for some reason an important priority in neural membranes. What is the cause of such specificity in membrane composition ? It is understood that biological membranes, while always having the form of a fluid lipid bilayer, have detailed distributions of lipid and protein molecules that reflect the interactions between lipids and integral membrane proteins [40]. It seems that the one missing double bond in DPA species renders them unsuitable for whatever lipid-protein interaction favours DHA's inclusion in membranes of the brain. Tight regulation of membrane lipid composition extends to differentiation between polyunsaturated species. We recently investigated the relationship between DHA and AA levels in plasma of red cell membranes of maternal and fetal blood samples. While these studies revealed only a modest correlation in levels of the two PUFA species in plasma choline phosphoglycerides ($r = 0.62$, $p < 0.001$, $n = 74$), a strong positive correlation was revealed between DHA and AA in the maternal red cell membrane ($r = 0.85$, $p < 0.0001$, $n = 74$), and a still tighter relationship in the red cells of preterm infants ($r = 0.88$, $p < 0.00005$, $n = 24$) [41]. Bearing in mind the very different dietary origins of these two PUFAs, such significant correlations indicate that some powerful mechanism exists to regulate their relative abundances in the membrane. It is possible that specific esterification processes could explain the correlations. The ethanolamine (PE) and serine phosphoglycerides (PS) have the highest content of DHA. In the brain there is an active

base-exchange reaction for serine and ethanolamine. Other w3 fatty acids do not esterify easily with PE and PS. So specificity of composition could be brought about by DHA-PS or DHA-PE formation. None-the-less the double bonds in positions 4-5 and 19-20 would still have to be relevant for the esterification to explain why the w6 and w3 DPAs might not match these conditions. Nuclear magnetic resonance (NMR) and fluorescence studies have attempted to differentiate the membrane properties conferred by PUFAs. In another paper [42], we discuss some of the constraints of such approaches and review the results obtained to date. Holte *et al.* [43] have conducted a thorough NMR investigation of the effects of polyunsaturation on lipid acyl chain orientational order, which revealed significant changes as the number of double bonds increased from one to three, but little difference as further double bonds were introduced. Ehringer *et al.* [44] directly compared the effects of 18 :3 and 22 :6 on membrane physical properties, and observed considerably higher permeability and perhaps vesicle fusability in the samples containing DHA.

In summary, a number of studies have been conducted on the physical effects of polyunsaturation on membranes, in which DHA has been compared to a range of other unsaturated chains having from one to five double bonds. Thus far, however, all differences that have been measured have been matters of degree, and none provide a compelling explanation for the striking specificity with which DHA is selected for membranes of the eye and brain. In addition, to our knowledge no study has compared DHA to either species of DPA to search for whatever property it is that causes neural membranes to discriminate so clearly between these seemingly similar molecules. The minimized energy structures presented here (see below) represent a preliminary step in this direction. Where, then, can we hope to find an explanation of DHA's preferred status in neural membranes ? An obvious starting point is in protein-lipid interactions : some way in which DHA favourably affects any of the myriad integral membrane proteins which are so important to neural membrane function. Such an effect could conceivably involve either a specific, molecular interaction between lipid and protein, or some modulation of bulk properties of the bilayer which alters protein function. We believe that specific binding interactions between lipid and protein molecules in a biological membrane are unlikely, since the membrane's fluid state means that individual lipid molecules will be undergoing rapid translational diffusion within the bilayer, and thus will never be in prolonged contact with any one protein. Furthermore, Brown's studies [45] on the rod photoreceptor outer segment membrane revealed that specific chemical-type interactions could not be the cause of DHA's established role in supporting rhodopsin function. It was found that full rhodopsin efficiency could be obtained by substituting other lipid mixtures designed to mimic the bulk mechanical properties of the physiological, DHA-rich membrane. This gave rise to a model in which DHA's role was to promote mechanical conditions in the membrane suitable to stabilize certain critical conformational changes undergone by rhodopsin in the course of photoactivation. These models do not fully reconstitute the structure of the photoreceptor cell and its synaptic function, the ten thousand fold adaptive capability of which is still unexplained. However, should this model be valid to conditions *in vivo* it could potentially be extended to other G-protein systems elsewhere in the CNS.

Applying this reasoning to the problem of distinguishing DHA from DPA, we must find a way in which the difference of one double bond might have a large enough impact on some bulk membrane property. The simulated structures shown in the figures are encouraging in this respect, as they show considerable differences between the minimized energy conformations of di-DHA PE and di-DPA PE (perhaps the first results which show a difference of sufficient magnitude to account for Nature's

longstanding, clear discrimination between the two). It must be stated, though, that these simulations have been carried out in vacuum and report only the lowest energy state ; their applicability to lipid molecules in a fluid bilayer at physiological temperatures is thus open to question.
A more speculative, possibility is that DHA *in vivo* plays a more direct role in neuronal signalling, in which some special properties conferred on the membrane by DHA chains exert an influence on membrane electrical phenomena. These might include distinctive dielectric or polarizability properties arising from the unique periodic and symmetric arrangement of double bonds in the DHA chain (which would be disrupted in DPAs). It is conceivable that some polarization of p-electron clouds might occur, and perhaps even be transmitted from one double bond to another (either within a given chain, or between neighbouring chains in the membrane). It must be emphasized that this model is strictly speculative, and there is no evidence, experimental or theoretical, to support it. An experiment to measure the dielectric response of lipid systems at a broad range of applied frequencies is currently being developed. In a similar vein, Penrose [46] has postulated that some brain functionality may arise due to quantum coherence in the microtubules of neurones [46] ; it may be worthwhile to look for a similar phenomenon in membranes containing DHA.

Global three dimensional energy minimized structures of DHA, n-3 DPA, n-6 DPA and various phospholipids containing these LC-PUFA were constructed with MOPAC software (Alchemy 2000 v. 2.0, Tripos Inc., St. Louis, MO). MOPAC (molecular orbital pacakage) which calculates the steric energy and energy minimized configuration of a given molecule by successive approximation, and is considered to be reasonably accurate as compared to known structures. The free fatty acids (FFA) DHA, n-3 DPA, n-6 DPA are shown in *figures 1 to 3*, respectively. The sixth ethylenic bond in DHA changes the character of the FFA, completing the methylene interupted sequence along the carbon chain, and conferring a folded, slightly spiral nature to the molecule. In n-3 DPA, the side of the chain closest to the terminal methyl is essentially ethylenic, while the other side is essentially saturated. The opposite is seen in the n-6 DPA, where the side of the chain closest to the methyl group is saturated, and the other side unsaturated. The DPAs lack the full methylene interupted sequence of double bonds throughout the carbon chain, which could be the basis of why they apparently do not have the functionality required by retinal and brain tissue.

The energy minimized ethanolaminephosphoglyceride structures with DHA *(figure 4)* and n-3 DPA dramatically illustrated the significance of the missing double bond in DPA *vs.* DHA. The final (C19) double bond in DHA constrains the position of the phosphoethanolamine head group, pulling it in and maintaining the spiral structure. This reduces the molecular volume, and may facilitate communication between the head group and the esterfied lipid chains. In contrast, the head group in n-3 DPA is far less constrained, and in fact moves away from the lipid ester chains. This structure would be more typical of phospholipids in general since most FA are less polyunsaturated than DHA. The cell membrane is in constant fluid motion so these structures only represent the preferred orientations of the molecules.x.

Conclusion

There is much still to be learned about the physical properties of membranes containing DHA. The extremely high degree of specificity with which it is selected for membranes of the brain (and has been, since very early evolutionary times) cannot be explained on the basis of the conventional

measurements that have been made thus far. DHA's special role may relate either to undefined interactions with integral membrane proteins or, more speculatively, to some role in neuronal signalling arising from unusual electrical properties. Nature's sharp discrimination between DHA and the nearly identical DPA species may give guidance to further inquiries into DHA's putative role, by focusing attention on the importance of the full complement of six periodic double bonds.

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Illustrations

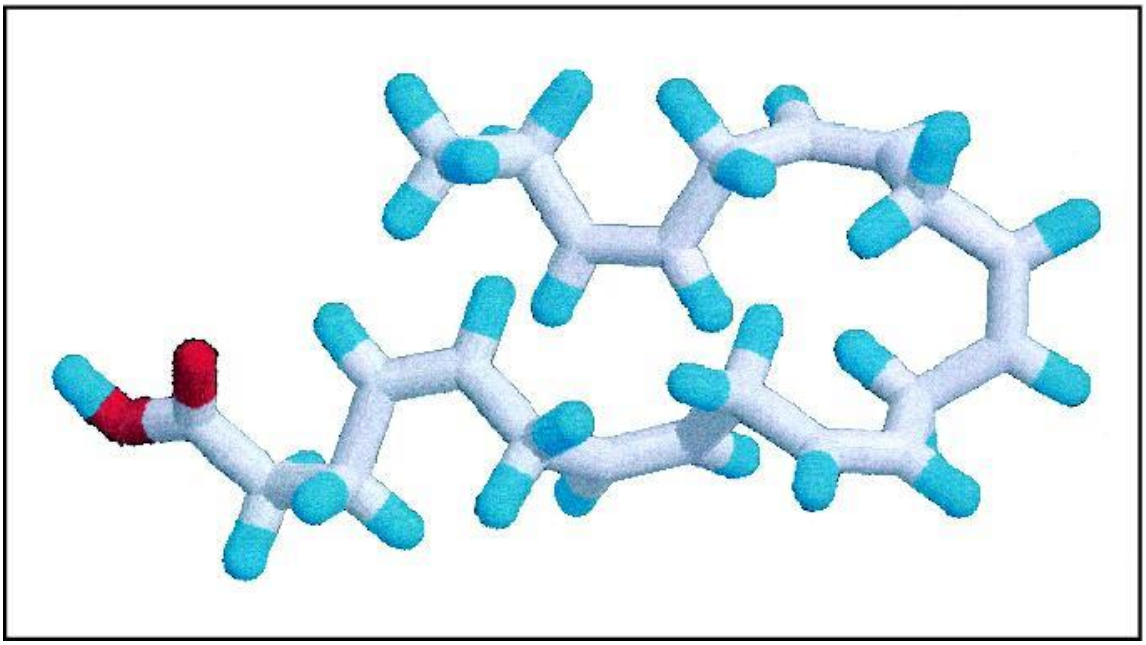

Figure 1. *3D energy-minimized structure of docosahexaenoic acid (DHA). This and following figures energy minimized and drawn with MOPAC as described in text.*

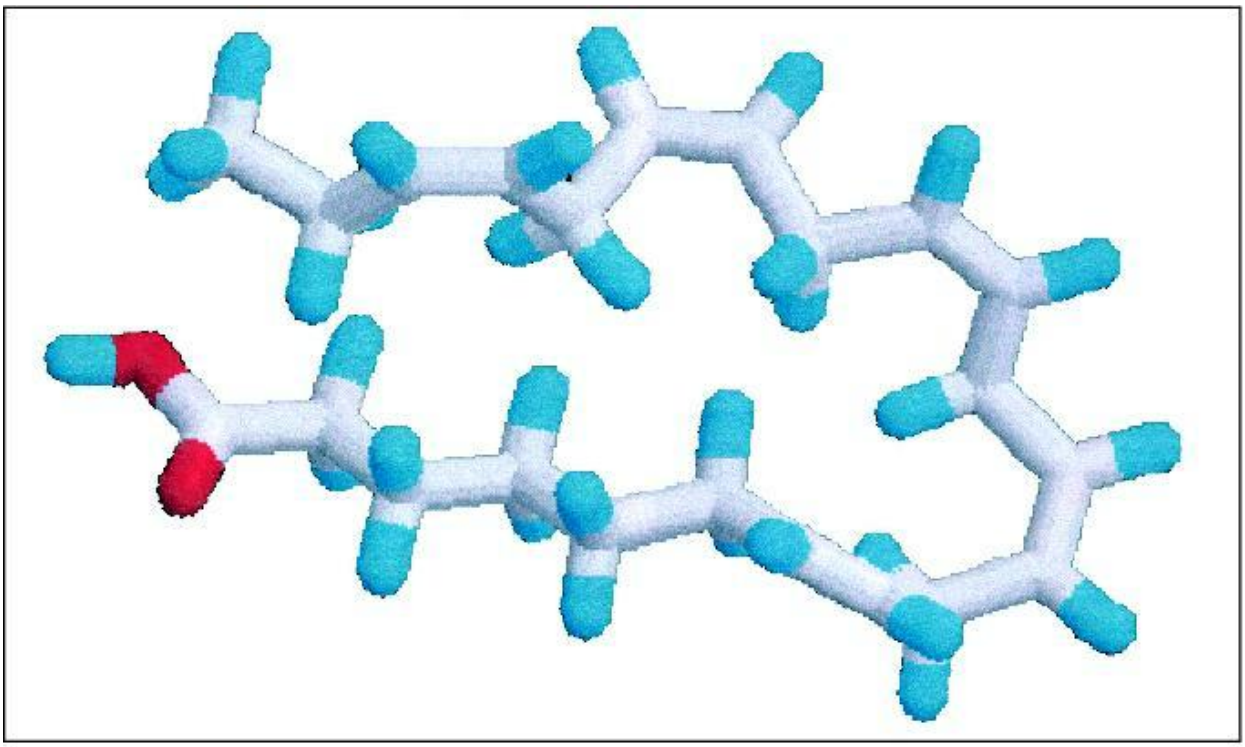

Figure 2. *N-3 docosapentaenoic acid (n-3 DPA).*

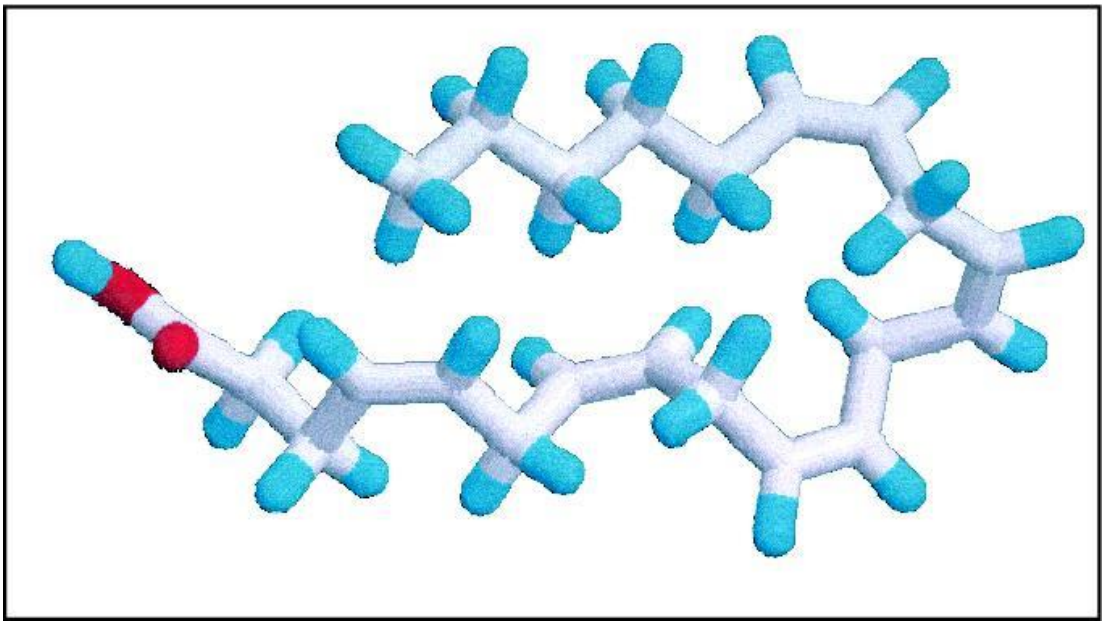

Figure 3. *N-6 docosapentaenoic acid (n-6 DPA).*

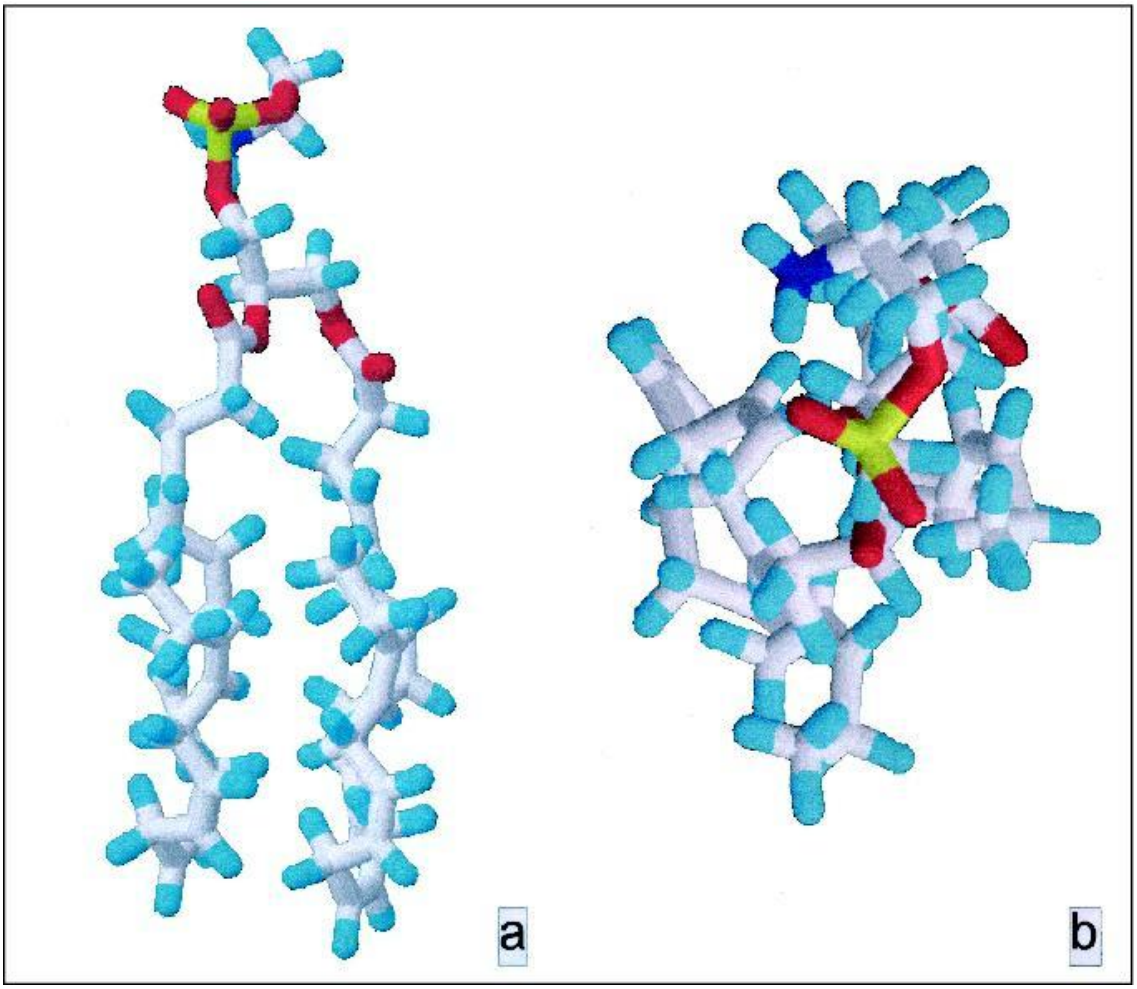

Figure 4. *a : 3D energy-minimized structure of phospholipid with ethanolamine, DHA, DHA. Side view. b : Ethanolamine, DHA, DHA. End view, note position of phosphate group.*